

Indole is a very important biological molecule being produced by more than 85 Gram positive and Gram negative bacterial species, including *Escherichia coli*. Among its several physiological functions indole is shown to stop bacterial cell division when secreted in large quantities.

Here we study the effect of indole on the electrical properties of the lipid membranes. Using electrophysiology, we show that the indole molecules are able to short-circuit lipid membranes. We also show that the rate of mitochondrial oxygen consumption measured in the presence of succinate increases when the indole concentration is titrated up. Therefore indole is capable of lowering the energetic barrier for ion permeation across lipid membranes. Since the bacterial cell division is strongly influenced by the ability of the cell to maintain a membrane potential(1) this is the first example of a bacterium using an ionophore to regulate the bacterial cell division. These findings have implications for our understanding of membrane biology, bacterial cell cycle control and potentially for the design of antibiotics that target the cell membrane.

1. Strahl, H. & Hamoen, L.W. Membrane potential is important for bacterial cell division. *Proceedings of the National Academy of Sciences* 107, 12281-12286 (2010).

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Triggered Gene Expression in Fed-Vesicle Microreactors with a Multifunctional Membrane

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We established a method to produce lipid vesicles encapsulating a DNA template and a minimal gene expression system for mRNA and protein synthesis. Our method is compatible with a variety of natural and functionalized lipids, which allowed us to engineer the vesicle membrane for surface immobilization and for promoting selective exchange with the environment. We demonstrated that gene expression could be triggered by supplying the nutrients - amino acids, nucleotides - as well as tRNAs in the outside solution. Protein synthesis can take place in liposomes having a broad range of sizes and the level of expression varies markedly between individual vesicles, a phenomenon resembling the stochastic nature of gene expression in cells. Moreover, we found that the transcription/translation lifetime could be extended from 2-3 hours in batch mode reactions until > 20 hours in liposome microreactors, likely by combined effects of the efflux of toxic reactional side products and the uptake of external resources.

We believe that our results are of direct relevance for initiating, regulating and monitoring biochemical reaction networks in general, with far-reaching consequences to the construction of an artificial cell and next generation drug delivery systems.

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Membrane Trafficking and Mechanics of the Golgi Apparatus: An in Cellulo Study by Optical Micromanipulation

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Membrane transport is based on the formation of tubulo-vesicular intermediates traveling from one compartment of the cell to another along cytoskeletal tracks. *In vitro* studies have shown that physical parameters, such as membrane curvature, tension and composition, influence the budding and fission of transport intermediates. A recent study in cells has highlighted the central role of the actin cytoskeleton in the fission of Rab6-positive transport intermediates from the Golgi apparatus (Miserey-Lenkei et al. 2010). Here we investigate the role of a mechanical stress on intracellular transport *in cellulo*. We focus on the mechanics of Golgi membranes and the formation of transport intermediates from the Golgi apparatus. Using confocal microscopy, we visu-

alize the deformation of Rab6-positive Golgi membranes applied by an internalized microsphere trapped in an optical tweezers, and simultaneously measure the corresponding forces. Our results show that the force necessary to deform Golgi membranes drops when the actin cytoskeleton is depolymerized, suggesting that actin strongly contributes to the local rigidity of the Golgi apparatus. We also show that the applied stress has a long-range effect on Golgi membranes and induces a sharp decrease in the formation of vesicles from the Golgi apparatus.

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Interfacing Natural and Artificial Proteins with Electronic Devices

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Integration of modern nanoelectronic technology with the potent molecular machines of living organisms provides opportunities to engineer controlled energy transfer in catalysts, solar cells, chemical sensors, and optoelectronics. To enable the production of bioelectronic devices that combine the functionalities of biomolecules with inorganic components, we are investigating properties and assembly of proteins at conductive interfaces. Since many of the key cellular functions are accomplished through system of enzymes and redox carrier molecules that are very complicated and unstable, we have adopted a different approach: we are reproducing the functional protein features within smaller, simpler, more robust model proteins, maquettes. To test the functionality, we have developed a facile method to covalently attach polyhistidine-tagged natural proteins and maquettes to graphene field effect transistors (FETs), nanotubes and highly ordered pyrolytic graphite. Atomic Force Microscopy and Raman spectroscopy are used to gain a structural understanding of the protein-carbon interface, while current-gate voltage measurements are used to elucidate the electronic properties. We will demonstrate how these electronic devices detect changes in light-activated proteins, including green and yellow fluorescent protein and Zn-protoporphyrin IX and flavin containing maquettes.

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Polystyrene Beads as a Model System for Virus Particles Reveal Pore Substructure as they Translocate

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Our research focuses on the extension of the resistive-pulse or Coulter counter technique to detect and analyze virus particles. Using polystyrene spheres as a model system, we show that particles can be distinguished not only by their size, but also by their charge, as they translocate through track-etched polyethylene terephthalate (PET) pores. In addition, we discovered that as a polystyrene sphere translocates, it reveals variations in the pore structure along its length as a series of peaks and valleys in the measured ionic current. This sequence of peaks and valleys is unique to a particular pore, but for that pore, we observed the same sequence for thousands of particle translocations, even particles of different size or with different charge. Also, particles translocating in the reverse direction through the pore give the reverse sequence of peaks and valleys in the ionic current.

We analyzed this data to extract information about the particle velocity as it passes through the pore and examined how this effect could enhance virus sensing capabilities. For example, due to the unique pattern of peaks and valleys, it is possible to unambiguously detect when two particles are in the pore at the same time. In addition, we expect that this characteristic pattern will be modulated by the particle geometry. Therefore, it will likely be possible to distinguish between particles of the same size but different shapes. After these model system studies, we plan to test our system with actual virus particles.